Effect of Laser Irradiation of Donor Blood on Erythrocyte Shape

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Changes in erythrocyte shape in donor blood during storage and after irradiation with He-Ne laser and infrared laser were studied by scanning electron microscopy, thick drop express-method, and morphometry. It was found that laser irradiation delayed the appearance of erythrocytes of pathological shapes (echinocytes, stomatocytes, *etc*.) in the blood; He-Ne laser produced a more pronounced effect.

Key Words: *erythrocytes; helium-neon and infrared laser*

Laser exposure of the blood (intravenous irradiation) was first applied by E. N. Meshalkin and V. S. Sergievskii [5]. Since then the intravascular (intravenous) laser irradiation of the blood (IVLIB) together with other methods of laser therapy became a routine tool of modern medicine [3,4,6,9,11]. Originally, He-Ne lasers were used for IVLIB. This is a complex procedure related to repeated use of special light guides. The appearance of infrared semicondicting (gallium arsenide) lasers and magnetic infrared lasers enabled transcutaneous laser irradiation of the blood. However, long-term experience of clinicians and researchers suggests that IVLIB with He-Ne laser is more effective due to coincidence of He-Ne laser wavelength and absorption spectrum of catalase, the main erythrocyte enzyme [2,4,6].

The development and introduction of special devices for IVLIB equipped with disposable needles, light guides, and a set of radioators with different wavelengths and powers made the IVLIB procedure simple and safe. Moreover, the use of different IVLIB modes with varying irradiation wavelength and power became possible [4].

Discocytes are predominant erythrocyte shape in the peripheral blood; this shape determines their high plasticity and possibility of passage through capillaries with diameter lower than discocyte diameter. However, the blood contains also pathological types of erythrocytes: echinocytes (erythrocytes with processes), stomatocytes (erythrocytes with impressions), *etc*. Various pathological states are associated with the increase in the count of pathological types of erythrocytes and a decrease in discocyte content [2,7,11,12].

Changes in the ratio of discocytes and pathological types of erythrocytes during storage of donor blood and after exposure to laser irradiation in different modes were never studied. Here we studied the effect of laser irradiation of donor blood on the content of pathological types of erythrocytes and discocyte integrity.

MATERIALS AND METHODS

Preserved donor blood stored for 1-11 days under standard conditions was used in the experiments. In experimental series I, blood samples (6 bags) were not irradiated (control). In series II and III, plastic bags with the blood were daily irradiated (10 min per day, scanning mode) using a Matriks-VLOK laser apparatus with different radiators. In series II, KL-VLOK-IK radiator with a wavelength of 0.808 μ (infrared irradiation) and output power of 35 mW was used. In series III, KL-VLOK-M radiator with a wavelength of

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0.63 μ (an analogue of He-Ne laser, red irradiation) and output power of 8 mW was applied.

Light microscopy of the blood was performed using thick drop express-method (TDEM) [8]. Two drops of the blood were fixed for 3-4 min in 1 ml 2.5% glutaraldehyde. Then, a drop of fixed blood was placed onto a slide and covered with a coverslip; the formed thick drop was analyzed under a light microscope equipped with a digital camera.

Erythrocytes of different shape were counted at ×600 (at least 1000 erythrocytes at each term and in each series were analyzed); photo images stored and

TABLE 1. Changes of Donor Blood Erythrocytes at Different Storage Terms (%)

Day of storage	Series	Discocytes	Echinocytes	Stomatocytes	Irreversible forms
Fresh blood		85.0±1.0	12.7±0.38	2.1±0.1	0.20±0.06
Day 1	I	80.1±1.1	18.2±0.1*	0.5±0.2*	1.2±0.3*
	II	82.6±1.6	13.9±1.5	2.4±0.1^{+}	1.1±0.1*
	III	85.2±1.6	12.9±1.5^{+}	1.4±0.7^{o}	0.5±0.1*^{o}
Day 2	I	75.8±2.0	2.7±0.9*	1.3±0.1*	2.0±0.1*
	II	81.5±1.6	15.6±1.8^{+}	1.7±0.1*	1.2±0.5*
	III	84.6±1.6	12.4±1.8^{+}	1.4±0.1*	1.6±0.1*
Day 3	I	63.4±2.0*	31.1±2.0*	2.3±0.4*	3.0±0.4*
	II	73.2±0.7	23.9±0.4	0.9±0.2^{+}	2.2±0.2^{+}
	III	80.4±0.7^{+}	17.4±0.4^{+}	1.2±0.2^{+}	1.0±0.2*^{o}
Day 4	I	61.0±2.0*	33.4±2.1*	2.6±0.4	3.9±0.5*
	II	68.5±2.2	28.3±1.2	1.5±0.3^{+}	1.7±0.3^{+}
	III	78.4±2.0^{+}	18.2±1.1^{o}	1.4±0.2^{+}	2.0±0.4*
Day 5	I	53.8±1.7*	38.5±1.7*	3.1±0.3*	4.6±0.5*
	II	62.5±0.9	33.2±0.5	1.9±0.1^{+}	2.4±0.1^{+}
	III	72.4±0.8	23.3±0.4^{o}	1.8±0.1^{+}	2.5±0.1^{+}
Day 6	I	44.1±2.4*	45.5±2.4*	4.8±0.4*	5.6±0.9*
	II	56.5±2.4	38.9±1.9^{+}	2.1±0.2^{+}	2.5±0.1^{+}
	III	68.4±2.4^{+}	27.3±1.9^{o}	1.9±0.1^{+}	2.4±0.1^{+}
Day 7	I	39.9±1.9*	47.4±2.0*	5.6±0.5*	7.1±1.2*
	II	49.4±1.1^{+}	43.1±1.2	3.3±0.1^{+}	4.2±0.6^{+}
	III	59.20±1.2	35.5±1.0^{o}	2.2±0.1^{+}	3.1±0.8^{+}
Day 8	I	28.3±1.7*	56.3±2.1*	6.2±0.6*	9.1±1.3
	II	40.6±1.8^{+}	49.6±1.8	3.9±0.1^{+}	5.9±0.5^{+}
	III	54.2±1.8^{o}	38.4±1.8^{o}	2.6±0.1^{+}	4.8±0.5^{+}
Day 9	I	19.0±1.7*	61.2±0.3*	9.6±0.4*	14.0±1.2*
	II	34.3±1.9^{+}	52.1±0.8	4.3±0.3^{+}	9.3±0.2^{+}
	III	54.0±1.6^{o}	40.3±0.7^{o}	2.7±0.3^{o}	3.0±0.2^{o}
Day 10	I	16.20±1.7*	52.2±1.7*	12.1±1.1*	19.3±1.7*
	II	32.2±1.2^{+}	44.3±0.6	9.5±0.4^{+}	14.0±0.6*
	III	52.4±1.2^{o}	40.2±0.6^{+}	4.5±0.4^{o}	3.4±0.6^{o}
Day 11	I	11.1±0.8*	60.7±2.0*	17.8±0.1*	10.4±1.6*
	II	30.7±0.9^{+}	35.2±0.3^{+}	15.6±0.5*	18.5±0.2^{+}
	III	50.4±0.8^{o}	42.4±0.4^{+}	4.2±0.4^{o}	3.0±0.1^{o}

Note. *p<0.05 in comparison with: *fresh blood, $^{+}$series I, ^{o}series II.

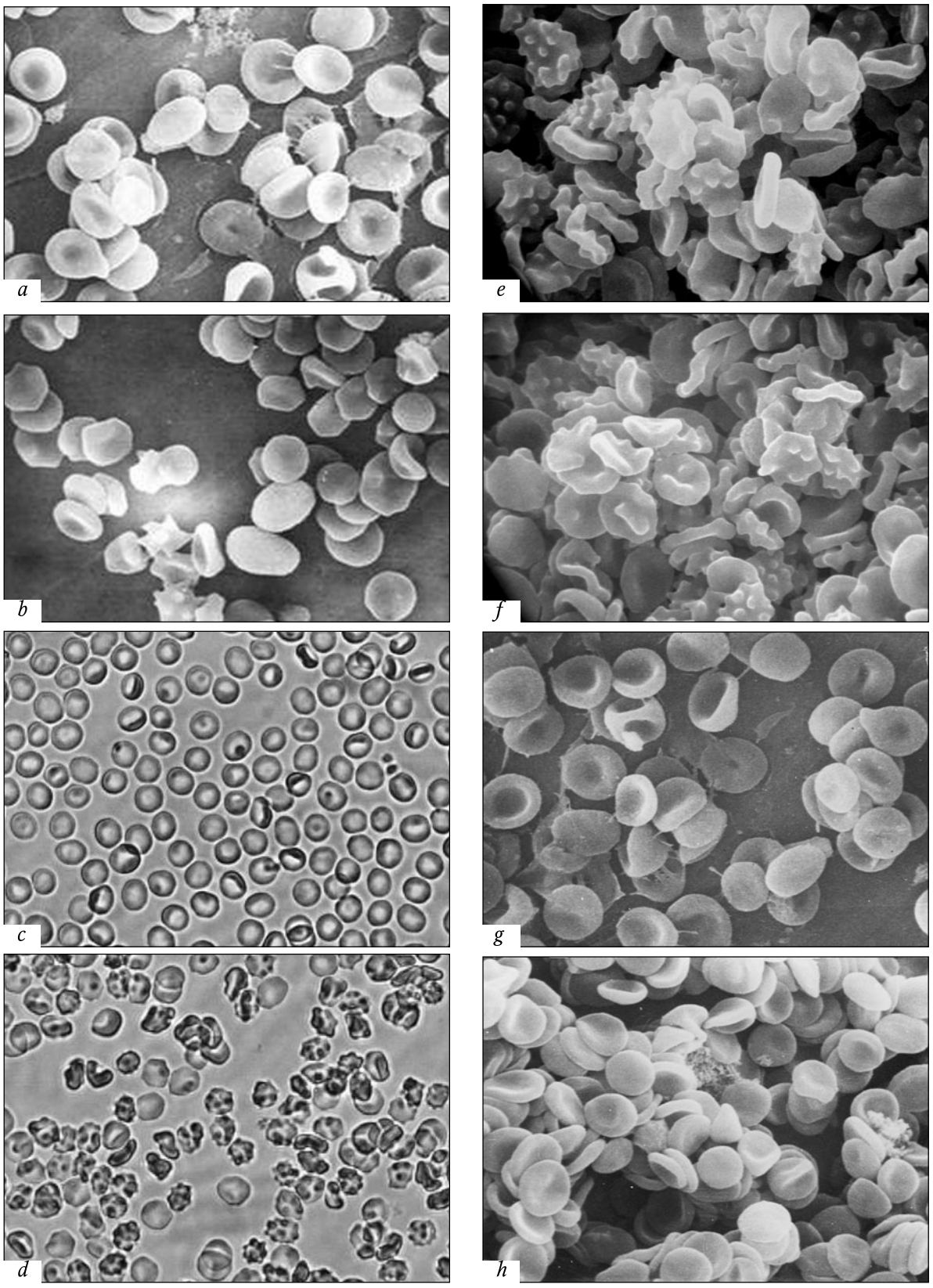

Fig. 1. Morphological examination of preserved donor blood. *a* (SEM, ×2000) and *c* (TDEM, ×600) – fresh blood: discocytes predominate. *b* (SEM, ×2000) and *d* (TDEM, ×600) – day 6 of storage (series I): predominance of pathological erythrocytes. *e* (SEM, ×2000) – day 7 of storage (series I): predominance of pathological erythrocytes. *f* (SEM, ×2000) – day 9 of storage (series I): predominance of pathological erythrocytes. *g* and *h* (SEM, ×2000) – day 9 of storage (series II and III, respectively): discocytes predominate.

processed on a computer. Erythrocytes were examined under Biolam-I2 and Axioskop 40 (Carl Zeiss) microscopes. Microphotographs were made on Axioskop 40 equipped with a digital camera coupled to a computer.

For scanning electron microscopy (SEM), the blood was fixed in 1 ml 2.5% glutaraldehyde in phosphate buffered saline (pH 7.4), dehydrated in ascending alcohols and acetone, and dried in a HCP-2 drier (Hitachi). The samples were mounted on aluminum matrix with electroconductive glue, coated with gold in an IB-3 apparatus (Eiko), and examined in a Hitachi S-405 A scanning electron microscope.

RESULTS

The content of pathological erythrocytes in preserved donor blood increased during storage (Fig. 1, *a, b, e*). The count of discocytes decreased and the content of echinocytes increased as soon as after 24 h. These differences were most pronounced on days 2-3 of storage (Table 1). The next day was characterized by further decrease in the content of discocytes and accumulation of pathological erythrocytes. On day 6, the summary content of echinocytes and stomatocytes surpassed that of discocytes, which can be regarded as critical changes because even the count of echinocytes surpassed that of discocytes (Fig. 1, *a-f*). On days 10 and 11, pathological erythrocytes predominated in the blood.

In series II (infrared irradiation, 0.808 μ), better stability of erythrocytes was observed (Fig. 1, *g*; Table 1). The count of discocytes gradually decreased and the number of echinocytes, stomatocytes, and degeneratively changed erythrocytes increased throughout the observation period, but these changes were less pronounced than in the control series. In series III (red irradiation, 0.63 μ, analogue of He-Ne laser), erythrocytes of donor blood were even better preserved (Fig. 1, *h*; Table 1).

Thus, we found that storage of donor blood for more than 5 days results in predominance of pathological erythrocytes. Laser exposure (especially red laser, 0.63 μ, analogue of He-Ne laser) ensures better integrity of donor blood erythrocytes.

On the basis of experimental studies of the effect of laser irradiation of erythrocyte deformability and optical properties of erythrocyte suspension, a model of erythrocyte response to laser exposure was proposed. The first stage consists in generation of singlet oxygen. Further stages are characterized by inactivation of excited oxygen molecules and structure and changes in biomembranes [1].

Irradiation of the blood with low-intensity laser is accompanied by the release of Ca^{2+} ions. The increase in Ca^{2+} concentration leads to cell activation. Erythrocyte shape is determined by spectrin, an inner membrane protein. The changes in erythrocyte shape can be explained by calcium-induced aggregation of spectrin [2,4,6].

He-Ne laser irradiation of bags with donor blood considerably improves rheological properties of the blood; therefore laser exposure can be used for improving preservation of donor blood [11]. Our experiments demonstrated that the structural basis of blood preservation is the decrease in the content of pathological erythrocytes and increase in discocyte content.

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